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ORIGINAL ARTICLE

# Copper(II) catalysis for oxidation of L-tryptophan by hexacyanoferrate(III) in alkaline medium: A kinetic and mechanistic approach

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Catalysis

**Abstract** The catalytic effect of copper(II) catalyst on the oxidation of L-tryptophan (Trp) by hexacyanoferrate(III) (HCF), has been investigated spectrophotometrically in an aqueous alkaline medium at a constant ionic strength of 0.5 mol $dm^{-3}$ and at 25 °C. The stoichiometry for both the uncatalyzed and catalyzed reactions was 1:2 (Trp:HCF). The reactions exhibited first order kinetics with respect to [HCF] and less than unit orders with respect to [Trp] and [$OH^-$]. The catalyzed reaction exhibited fractional-first order kinetics with respect to [$Cu^{II}$]. The reaction rates were found to increase as the ionic strength and dielectric constant of the reaction medium increase. The effect of temperature on the rates of reactions has also been studied, and the activation parameters associated with the rate-determining steps of the reactions have been evaluated and discussed. Addition of the reaction product HCF(II) to the reaction mixture did not affect the rates. Plausible mechanistic schemes for uncatalyzed and catalyzed reactions explaining all of the observed kinetic results have been proposed. In both cases, the final oxidation products are identified as indole-3-acetaldehyde, ammonia, and carbon dioxide. The rate laws associated with the reactions' mechanisms are derived. The rate constants of the slow steps of the reactions along with the equilibrium constants are also calculated.


## 1. Introduction

Numerous kinetic investigations of the oxidation of amino acids have been performed in recent decades using various oxidants under different experimental conditions [1–9] because of their biological significance, selectivity toward oxidants and bearing on the mechanism of amino acid metabolism. In many cases, it was reported that amino acids undergo oxidative decarboxylation and deamination. L-Tryptophan (Trp) is an

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essential amino acid in the human diet. It finds extensive application as a reducing agent in chemical and biochemical systems. Oxidation of L-tryptophan has been studied in acid media by few reagents such as chromate [6], permanganate [7] and vanadium [8], and in alkaline media by diperiodatocuprate(III) [9].

Hexacyanoferrate(III) (HCF) is an efficient one-electron oxidant that has been observed to be inert to substitution [10]. Its usefulness is due to its high stability, water solubility and moderate reduction potential of 0.45 V during reduction to hexacyanoferrate(II), a stable product [11]. In addition, it adds less error to the experimental results, since data can be analyzed meticulously to establish the reaction path. The chemistry of hexacyanoferrate(III) in alkaline medium, particularly its oxidative capacity in oxidation of organic [10–15] and inorganic [16–19] compounds, is well understood.

A literature survey revealed that the kinetics and mechanism of oxidation of some amino acids by hexacyanoferrate (III) in alkaline media have been studied earlier [10–15]. It was observed that such reactions proceed very slowly in the absence of a catalyst, but they proceed more rapidly with the use of some metal ion catalysts. These catalyzed reactions follow complex kinetics. In fact, kinetic studies of the oxidation reactions of amino acids catalyzed by different metal ions are an important field of chemistry because of the role played by metals in biological systems. Although catalysis by transition metal ions depends on the nature of the substrate, oxidant, and experimental conditions, it has been reported [20,21] that metal ions act as catalysts by one of several different paths, such as the formation of complexes with reactants, oxidation of the substrate itself or through the formation of free radicals.

No work, however, has been reported on the oxidation of L-tryptophan by alkaline hexacyanoferrate(III) either in the absence or presence of any catalyst. The present report deals with the title reaction in order to determine the selectivity of L-tryptophan toward hexacyanoferrate(III) in an alkaline medium, to examine the catalytic activity of the copper(II) catalyst, to understand the kinetically active species of the reactants, to identify the reaction products and finally to propose appropriate reaction mechanisms.

## 2. Experimental

### 2.1. Materials

All chemicals employed in the present work were of reagent grade and their solutions were prepared by dissolving the requisite amounts of the samples in doubly distilled water. A stock solution of L-tryptophan was freshly prepared by dissolving the amino acid sample (E. Merck, UK) in bi-distilled water. A fresh solution of hexacyanoferrate(III) was prepared by dissolving potassium hexacyanoferrate(III) (BDH) in water, and its concentration was ascertained spectrophotometrically. Hexacyanoferrate(II) solution was prepared by dissolving potassium hexacyanoferrate(II) (S. D. Fine Chem.) in water and standardizing with cerium(IV) solution [22]. Sodium hydroxide and sodium perchlorate were used to vary the alkalinity and ionic strength of the reactions medium, respectively.

### 2.2. Kinetic measurements

Kinetic runs were carried out under pseudo-first order conditions with a large excess of L-tryptophan over hexacyanoferrate(III). The reactions were initiated by mixing the previously thermostatted solutions of HCF and Trp, which also contained the required amounts of NaOH and $NaClO_4$, and copper(II) catalyst in the case of catalyzed reaction. The progresses of both uncatalyzed and copper(II)-catalyzed reactions were followed by monitoring the decrease in the absorbances of HCF as a function of time. Absorption was monitored at a wavelength of 439 nm, rather than at $\lambda = 420$ nm (the absorption maximum of HCF), due to a bathochromic shift of about 19 nm upon mixing the reactants. The other constituents of the reaction mixtures did not absorb significantly

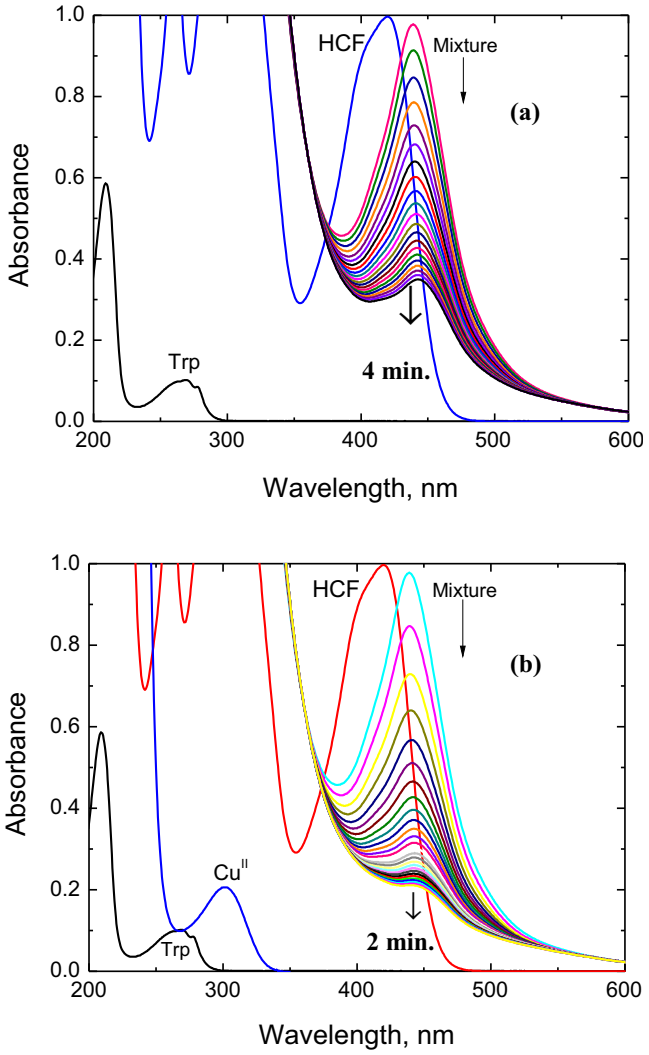



**Figure 1** Time-resolved spectra for: (a) uncatalyzed, and (b) copper(II)-catalyzed oxidation of L-tryptophan by hexacyanoferrate(III) in alkaline medium. [Trp] = 0.02, [HCF] = $7.0 \times 10^{-4}$, $[OH^-] = 0.2$ and $I = 0.5$ mol dm$^{-3}$ at 25 °C. $[Cu^{II}] = 5.0 \times 10^{-5}$mol dm$^{-3}$.

at this wavelength. The applicability of Beer's law for HCF at 420 nm has been verified giving $\varepsilon = 1048 \pm 17\ dm^3\ mol^{-1}\ cm^{-1}$ in a good agreement with the values reported elsewhere [23]. The absorbance measurements were made in a thermostatted Shimadzu UV–VIS-NIR-3600 double-beam spectrophotometer.

The reaction between hexacyanoferrate(III) and L-tryptophan in an alkaline medium proceeded with a measurable rate in the absence of copper(II) catalyst. The catalyzed reaction is understood to occur in parallel paths, with contributions from both the uncatalyzed and catalyzed reactions. Thus, the total rate constant ($k_T$) is equal to sum of the rate constants of the uncatalyzed ($k_U$) and catalyzed ($k_C$) reactions, such that: $k_C = k_T - k_U$.

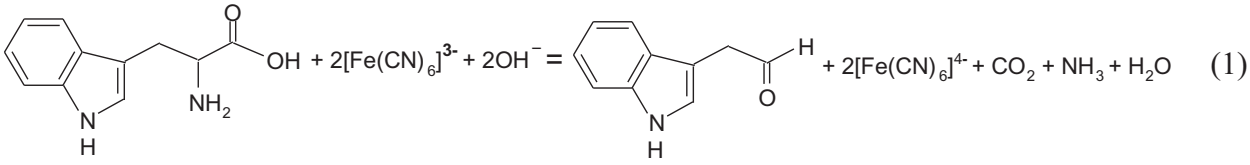
First order plots of log(absorbance) versus time were straight lines up to at least 85% completion of the reactions, and the pseudo-first order rate constants ($k_U$ and $k_C$) were calculated as the gradients of such plots. Average values of at least two independent measurements of the rate constants were taken for the analysis. The rate constants were reproducible to within 4%.

Several kinetic runs were carried out after bubbling purified nitrogen through the solutions and compared with those taken under air, and the results were the same. Thus, dissolved oxygen did not affect the oxidation rate.

## 3. Results

### 3.1. Stoichiometry and product analysis

The stoichiometry is determined spectrophotometrically at $[OH^-] = 0.2$ and $I = 0.5\ mol\ dm^{-3}$ indicating consumption of two HCF ions for one molecule of Trp to yield the oxidation products as shown in the following equation,




Tryptophan Indole-3-acetaldehyde

The product aldehyde, indole-3-acetaldehyde, is tested by thin layer chromatography. One spot is obtained when the chromatogram is assayed with 2,4-dinitrophenylhydrazine. An aliquot (5 mL) is pipetted into 50 mL of 2.0 N HCl saturated with 2,4-dinitrophenylhydrazine, which is filtered, washed, dried and weighed. The yield is calculated from the amount of 2,4-dinitrophenylhydrazone formed, and was about 84%. Further proof of the formation of the aldehyde is obtained by isolating the aldehyde using the standard method [24]. The byproducts are identified as ammonia and carbon dioxide by Nessler's reagent [25] and lime water, respectively. Similar oxidation products with different experimental conditions have been reported earlier [6–8].

### 3.2. Time-resolved spectra

Spectral scans during the oxidation of L-tryptophan by hexacyanoferrate(III) in alkaline medium are shown in Fig. 1a and b, in the absence and presence of copper(II) catalyst, respectively. In both cases, the scanned spectra indicate a gradual disappearance of the HCF(III) band with time as a result of its reduction to HCF(II). Bathochromic shifts in the HCF absorption band of about 19 nm from 420 to 439 nm are apparent in both spectra.

### 3.3. Reaction order

The reaction orders with respect to the reactants for both the uncatalyzed and catalyzed reactions are determined from the slopes of the log $k_U$ and log $k_C$ versus log(concentration) plots by varying the concentrations of substrate, alkali and catalyst, in turn, while keeping other conditions constant.

The concentration of the hexacyanoferrate(III) oxidant is varied in the range $3.0–12.0 \times 10^{-4}\ mol\ dm^{-3}$, while other variables such as the concentrations of the reductant, copper(II) catalyst and sodium perchlorate, and the pH and temperature are also kept constant. It is evident that the increase in the oxidant concentration did not alter the oxidation rates of L-tryptophan (Table 1). This indicates that the oxidation rates are independent of oxidant concentration, and confirms that the order of reactions with respect to the oxidant is 1.

The observed rate constants ($k_U$ and $k_C$) are determined at different initial concentrations of the reductant L-tryptophan, while maintaining other species at fixed concentrations. Plots of the observed rate constants versus [Trp] at constant pH were linear with positive intercepts (Fig. 2). These observations confirm that the dependences with respect to the amino acid are fractional-first order for both the uncatalyzed and catalyzed reactions.

The reaction rates are measured at constant [Trp], [HCF], [$Cu^{II}$] (for the catalyzed reaction), ionic strength and temperature, but with varying [$OH^-$] ($0.1–0.5\ mol\ dm^{-3}$). The rates of the reactions increased with the increasing [$OH^-$]. Plots of $k_U$ and $k_C$ versus [$OH^-$] are linear with positive intercepts, as shown in Fig. 3, confirming fractional-first order dependences with respect to [$OH^-$].

The copper(II) catalyst concentration is varied from $1.0 \times 10^{-5}$ to $9.0 \times 10^{-5}\ mol\ dm^{-3}$ at constant [Trp], [HCF], [$OH^-$], ionic strength and temperature. The reaction rate increased with increasing [$Cu^{II}$] (Table 1). An order of 0.87

**Table 1** Effect of variation of [HCF], [Trp], [$OH^-$], [$Cu^{II}$] and ionic strength, *I*, on the pseudo-first order rate constants in the uncatalyzed and copper(II)-catalyzed oxidation of L-tryptophan by hexacyanoferrate(III) in alkaline medium at 25 °C.

| $10^4$ [HCF] (mol $dm^{-3}$) | $10^2$ [Trp] (mol $dm^{-3}$) | [$OH^-$] (mol $dm^{-3}$) | $10^5$ [$Cu^{II}$] (mol $dm^{-3}$) | I (mol $dm^{-3}$) | $10^4 k_T$ ($s^{-1}$) | $10^4 k_U$ ($s^{-1}$) | $10^4 k_C$ ($s^{-1}$) |
|---|---|---|---|---|---|---|---|
| 3.0 | 2.0 | 0.2 | 5.0 | 0.5 | 41.12 | 9.41 | 31.71 |
| 5.0 | 2.0 | 0.2 | 5.0 | 0.5 | 43.09 | 9.58 | 33.51 |
| 7.0 | 2.0 | 0.2 | 5.0 | 0.5 | 42.20 | 9.50 | 32.70 |
| 9.0 | 2.0 | 0.2 | 5.0 | 0.5 | 42.39 | 9.61 | 32.78 |
| 12.0 | 2.0 | 0.2 | 5.0 | 0.5 | 42.11 | 9.40 | 32.71 |
| 7.0 | 0.5 | 0.2 | 5.0 | 0.5 | 22.22 | 5.12 | 17.1 |
| 7.0 | 1.0 | 0.2 | 5.0 | 0.5 | 30.94 | 7.15 | 23.79 |
| 7.0 | 2.0 | 0.2 | 5.0 | 0.5 | 42.20 | 9.50 | 32.70 |
| 7.0 | 3.0 | 0.2 | 5.0 | 0.5 | 52.00 | 11.45 | 40.55 |
| 7.0 | 4.0 | 0.2 | 5.0 | 0.5 | 61.01 | 13.29 | 47.72 |
| 7.0 | 2.0 | 0.1 | 5.0 | 0.5 | 29.44 | 6.12 | 23.32 |
| 7.0 | 2.0 | 0.2 | 5.0 | 0.5 | 42.20 | 9.50 | 32.70 |
| 7.0 | 2.0 | 0.3 | 5.0 | 0.5 | 54.31 | 12.74 | 41.57 |
| 7.0 | 2.0 | 0.4 | 5.0 | 0.5 | 65.24 | 16.21 | 49.03 |
| 7.0 | 2.0 | 0.5 | 5.0 | 0.5 | 74.41 | 19.38 | 55.03 |
| 7.0 | 2.0 | 0.2 | 1.0 | 0.5 | 14.62 | 9.50 | 5.12 |
| 7.0 | 2.0 | 0.2 | 3.0 | 0.5 | 27.50 | 9.50 | 18.00 |
| 7.0 | 2.0 | 0.2 | 5.0 | 0.5 | 42.20 | 9.50 | 32.70 |
| 7.0 | 2.0 | 0.2 | 7.0 | 0.5 | 53.53 | 9.50 | 44.03 |
| 7.0 | 2.0 | 0.2 | 9.0 | 0.5 | 64.41 | 9.50 | 54.91 |
| 7.0 | 2.0 | 2.0 | 5.0 | 0.5 | 42.20 | 9.50 | 32.70 |
| 7.0 | 2.0 | 2.0 | 5.0 | 0.7 | 49.55 | 10.91 | 38.64 |
| 7.0 | 2.0 | 2.0 | 5.0 | 1.0 | 63.24 | 12.33 | 50.91 |
| 7.0 | 2.0 | 2.0 | 5.0 | 1.5 | 83.15 | 14.87 | 68.28 |
| 7.0 | 2.0 | 2.0 | 5.0 | 2.0 | 99.27 | 16.21 | 83.06 |

Experimental error ±5%.

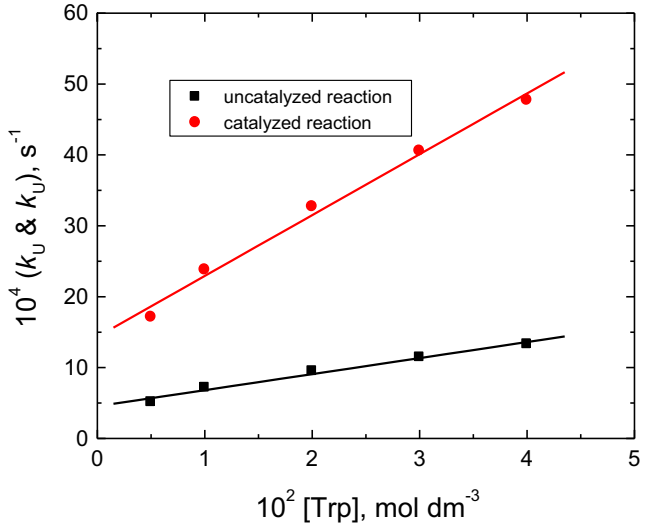



**Figure 2** Plots of $k_U$ and $k_C$ versus [Trp] in the uncatalyzed and copper(II)-catalyzed oxidation of L-tryptophan by hexacyanoferrate(III) in alkaline medium. [HCF] = $7.0 \times 10^{-4}$, [$OH^-$] = 0.2 and $I$ = 0.5 mol $dm^{-3}$ at 25 °C. [$Cu^{II}$] = $5.0 \times 10^{-5}$mol $dm^{-3}$ for the catalyzed reaction.

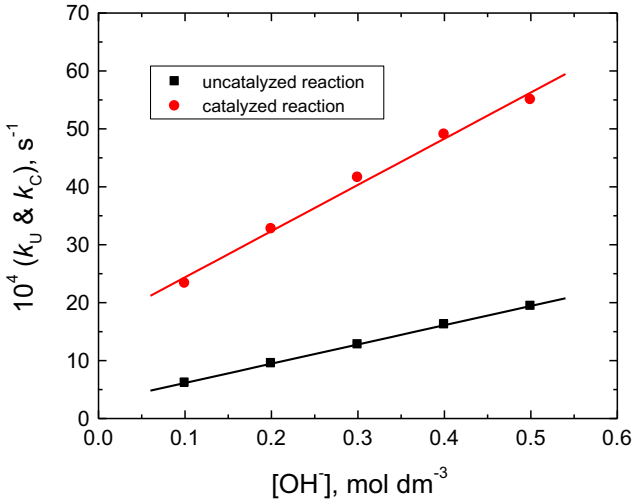



**Figure 3** Plots of $k_U$ and $k_C$ versus [$OH^-$] in the uncatalyzed and copper(II)-catalyzed oxidation of L-tryptophan by hexacyanoferrate(III) in alkaline medium. [Trp] = 0.02, [HCF] = $7.0 \times 10^{-4}$ and $I$ = 0.5 mol $dm^{-3}$ at 25 °C. [$Cu^{II}$] = $5.0 \times 10^{-5}$mol $dm^{-3}$ for the catalyzed reaction.

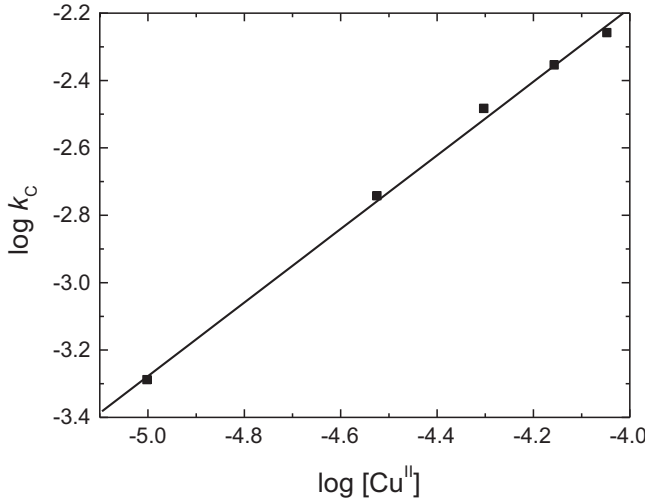



**Figure 4** A plot of log $k_C$ versus log [$Cu^{II}$] in the copper(II)-catalyzed oxidation of L-tryptophan by hexacyanoferrate(III) in alkaline medium. [Trp] = 0.02, [HCF] = $7.0 \times 10^{-4}$, [$OH^-$] = 0.2 and $I$ = 0.5 mol $dm^{-3}$ at 25 °C.

was obtained from the slope of the log $k_C$ versus log[$Cu^{II}$] plot (Fig. 4).

### 3.4. Effect of ionic strength and dielectric constant

To investigate the effect of ionic strength on both the uncatalyzed and catalyzed reactions, the reactions were studied at several initial concentrations of sodium perchlorate with constant concentrations of Trp, HCF and $Cu^{II}$, and at constant pH and temperature. The results are presented in Table 1. These results showed that the pseudo-first order rate constants ($k_U$ and $k_C$) increase with the increasing ionic strength of the medium, and the Debye–Hückel plots were linear with positive slopes as shown in Fig. 5a.

The effect of dielectric constant or relative permittivity, $D$, is studied by varying the $t$-butyl alcohol–water content in the reaction mixtures at 25 °C. The rate constants were found to decrease with decreasing dielectric constant of the solvent mixture, i.e. increasing $t$-butyl alcohol content. The plots of log $k_U$ and log $k_C$ versus $1/D$ were linear with negative slopes (Fig. 5b).

### 3.5. Effect of initially added product

The effect of addition of the product hexacyanoferrate(II) is also studied in the concentration range 3.0–12.0 × $10^{-4}$ mol $dm^{-3}$ at fixed concentrations of the oxidant, reductant, alkali and catalyst. HCF(II) had no significant effect on the rate of reaction.

### 3.6. Effect of temperature

To evaluate the activation parameters, the effect of temperature on the rates of both the uncatalyzed and catalyzed oxidation reactions is studied by performing the reactions at temperatures of 15, 20, 25, 30 and 35 °C, with reactant concentrations and other conditions held constant. The results obtained indicate that the pseudo-first order rate constants increased with rising temperature. The activation parameters of the second order rate constant, $k$, are calculated using Arrhenius and Eyring plots (Fig. 6a and b, respectively), and are listed in Table 2.

### 3.7. Polymerization test

The possible intervention of free radicals during the oxidation reactions was assayed by a polymerization test. Known amounts of acrylonitrile scavenger are added to reaction mixtures, which are kept for 6 h in an inert atmosphere. On dilution of the mixtures with methanol, white precipitates are formed, thus confirming the presence of free radicals

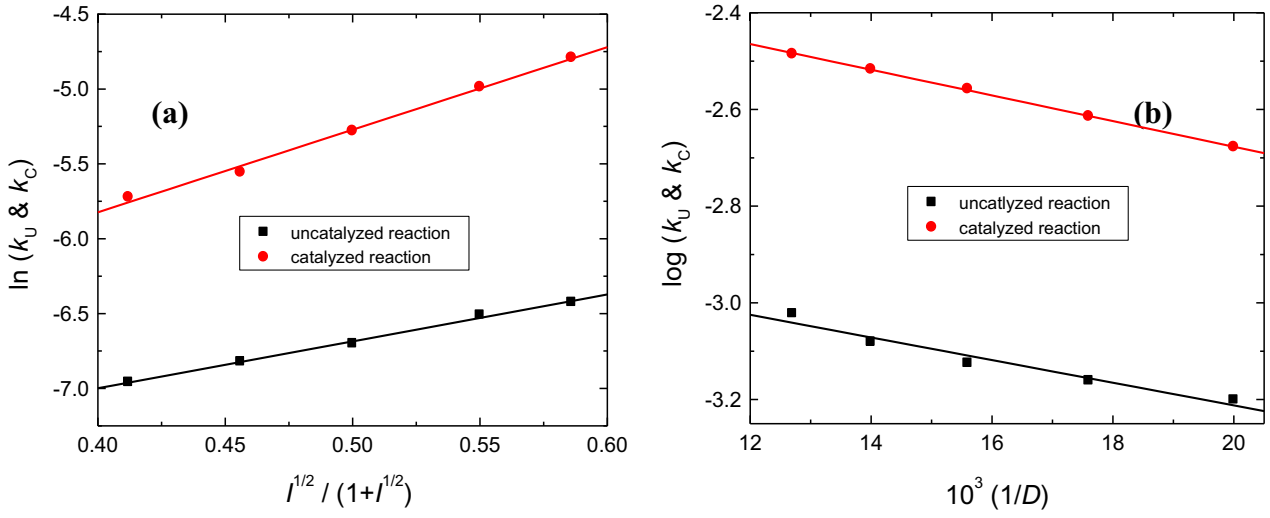



**Figure 5** Effect of (a) ionic strength, $I$, and (b) dielectric constant, $D$, of the medium on the uncatalyzed and copper(II)-catalyzed oxidation of L-tryptophan by hexacyanoferrate(III) in alkaline medium. [Trp] = 0.02, [HCF] = $7.0 \times 10^{-4}$ and [$OH^-$] = 0.2 mol $dm^{-3}$ at 25 °C. [$Cu^{II}$] = $5.0 \times 10^{-5}$ mol $dm^{-3}$ for the catalyzed reaction.

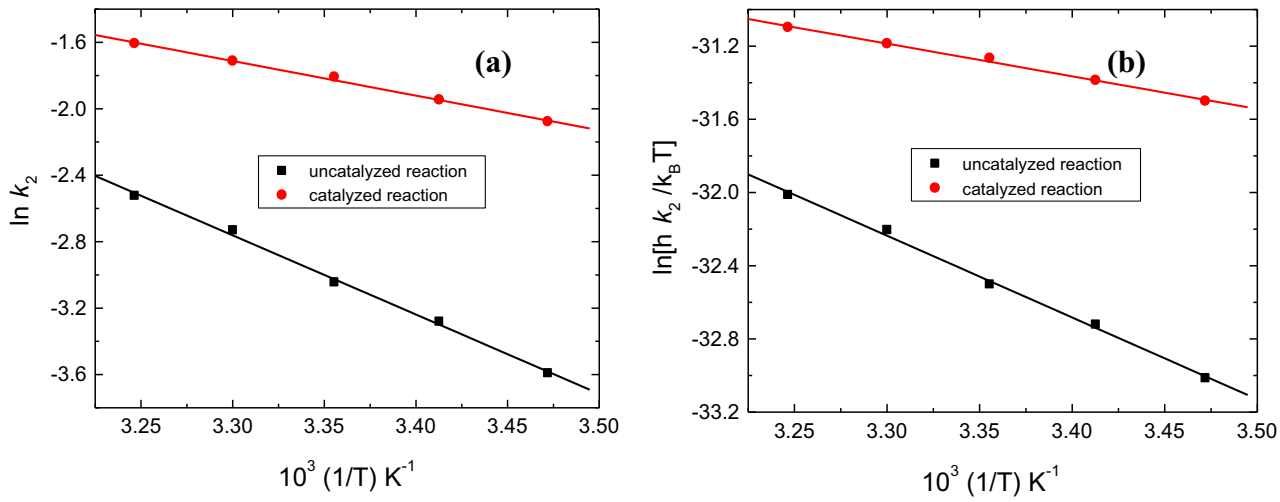



**Figure 6** (a) Arrhenius, and (b) Eyring plots in the uncatalyzed and copper(II)-catalyzed oxidation of L-tryptophan by hexacyanoferrate (III) in alkaline medium. [Trp] = 0.02, [HCF] = $7.0 \times 10^{-4}$, [$OH^-$] = 0.2 and $I$ = 0.5 mol $dm^{-3}$. [$Cu^{II}$] = $5.0 \times 10^{-5}$mol $dm^{-3}$ for the catalyzed reaction.

**Table 2** Activation parameters of $k$ in the uncatalyzed and copper(II)-catalyzed oxidation of L-tryptophan by hexacyanoferrate(III) in alkaline medium. [Trp] = 0.02, [HCF] = $7.0 \times 10^{-4}$, [$OH^-$] = 0.2 and $I$ = 0.5 mol $dm^{-3}$. [$Cu^{II}$] = $5.0 \times 10^{-5}$mol $dm^{-3}$ for the catalyzed reaction.

| Reaction | $\Delta S^{\neq}$, J $mol^{-1}K^{-1}$ | $\Delta H^{\neq}$, kJ $mol^{-1}$ | $\Delta G^{\neq}_{298}$, kJ $mol^{-1}$ | $E_a^{\neq}$, kJ $mol^{-1}$ |
|---|---|---|---|---|
| Uncatalyzed | −145.49 | 37.08 | 80.44 | 39.91 |
| Catalyzed | −209.93 | 14.38 | 76.94 | 17.29 |

Experimental error ±4%.

intervention in these reactions. When these experiments are repeated in the absence of L-tryptophan under otherwise similar conditions, the tests were negative. This indicates that the reactions proceeded via free radical pathways.

## 4. Mechanism of the uncatalyzed oxidation reaction

Hexacyanoferrate(III) oxidation of L-tryptophan in alkaline medium was found to occur at a slow rate in the absence of the copper(II) catalyst. The reaction had a stoichiometry of 2:1, i.e. two moles of hexacyanoferrate(III) reacted with one mole of L-tryptophan. The reaction exhibited first order dependence with respect to [HCF] and less than unit order with respect to [Trp]. The rate of HCF reduction increased with increasing [$OH^-$] with a fractional-first order dependence, suggesting deprotonation of L-tryptophan by the alkali prior to the rate-determining step that forms a more reactive species of the reductant [26]. The rate was not affected by the addition of HCF(II), indicating that the possibility of a fast equilibrium with the product preceding the rate-determining step can be ruled out. Therefore, the rate-determining step should be irreversible, as is generally the case for one-electron oxidants [27], and the oxidation takes place through generation of a free radical, as observed experimentally. In addition, the rate of reaction increased with the increase in the ionic strength and dielectric constant of the medium, suggesting that the reaction occurs between two similarly charged ions [28,29].

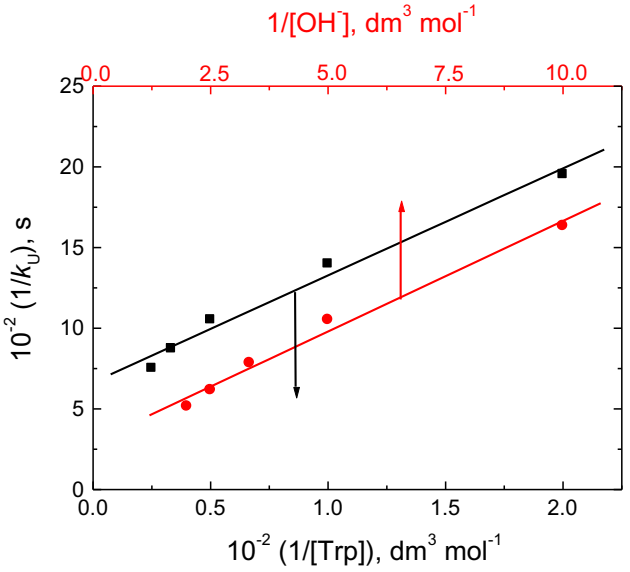
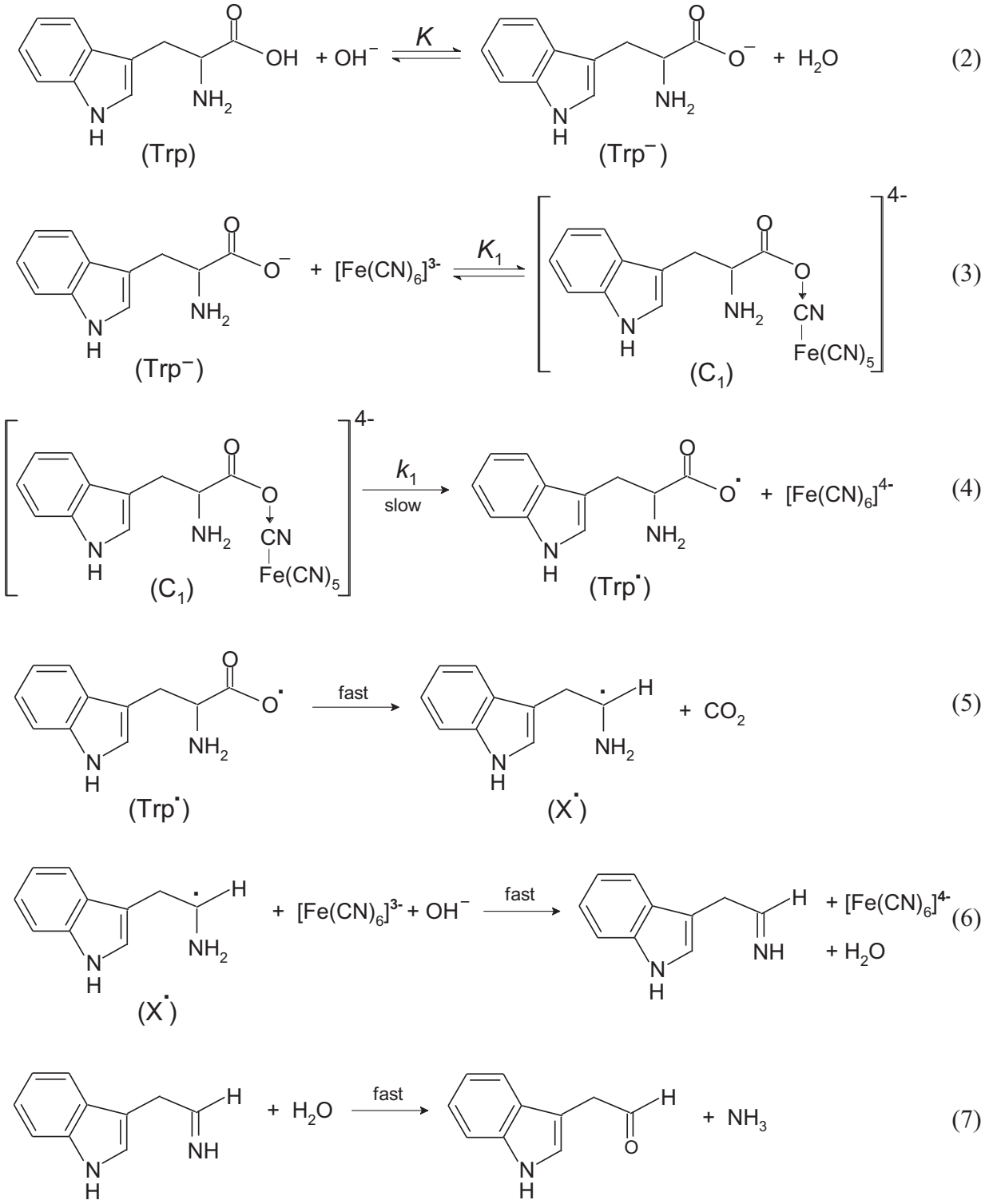



**Figure 7** Verification of Eqs. (11) and (12) in the uncatalyzed oxidation of L-tryptophan by hexacyanoferrate(III) in alkaline medium. [HCF] = $7.0 \times 10^{-4}$ and $I$ = 0.5 mol $dm^{-3}$ at 25 °C.

On the other hand, the less than unit order in [Trp] may be due to formation of a complex ($C_1$) between the HCF species and the deprotonated L-tryptophan species prior to the




**Scheme 1** Mechanism of uncatalyzed oxidation of L-tryptophan by hexacyanoferrate(III) in alkaline medium.

rate-determining step. Complex formation is well supported by spectroscopic evidence obtained from UV–Vis spectra (Fig. 1a) where a bathochromic shift of about 19 nm from 420 to 439 nm in spectral changes was observed. Additional spectral evidence [30] is the observed increase in the initial absorbance upon mixing of the reactants. Complex formation was also proved kinetically by the non-zero intercept of the plot $1/k_U$ versus 1/[Trp] (Fig. 7). The formed complex ($C_1$) is slowly decomposed in the rate-determining step to give rise to the initial oxidation products as the substrate intermediate radical ($Trp^{\cdot}$) and HCF(II). This is followed by decarboxylation of L-tryptophan free radical, forming a new radical intermediate

(X). This reacts with another HCF species in a subsequent fast step to give rise to the final oxidation products, as given in Scheme 1.

Furthermore, it has been reported [31,32] that the entropy of activation tends to be more negative for the reactions of inner-sphere nature, whereas reactions with positive $\Delta S^{\neq}$ values proceed via an outer-sphere mechanism. The obtained large negative values of entropy of activation (Table 2) suggest the inner-sphere mechanism for oxidation of Trp by alkaline HCF, where the transfer of an electron occurs from Trp to $Fe^{III}$ through a cyano bridging ligand. On the other hand, the positive values of $\Delta H^{\neq}$ and $\Delta G^{\neq}$ indicate that the complex formation is endothermic and non-spontaneous, respectively.

The suggested mechanism leads to the following rate law (see Appendix A),

$$\text{Rate} = \frac{k_1 K K_1[\text{Trp}][\text{HCF}][\text{OH}^-]}{1 + K[\text{OH}^-] + K K_1[\text{Trp}][\text{OH}^-]} \tag{8}$$

where [Trp], [HCF] and [OH] refer to the total (analytical) concentrations of L-tryptophan, hexacynoferrate(III) and alkali, respectively.

The above rate law is consistent with all observed orders with respect to different species.

Under the pseudo-first order condition, the rate-law can be expressed by Eq. (9),

$$\text{Rate} = \frac{-d[\text{HCF}]}{dt} = k_U[\text{HCF}] \tag{9}$$

Comparing Eqs. (8) and (9), the following relationship is obtained,

$$k_U = \frac{\text{Rate}}{[\text{HCF}]} = \frac{k_1 K K_1[\text{Trp}][\text{OH}^-]}{1 + K[\text{OH}^-] + K K_1[\text{Trp}][\text{OH}^-]} \tag{10}$$

Eq. (10) can be rearranged to the following forms, which are suitable for verification,

$$\frac{1}{k_U} = \left(\frac{1}{k_1 K K_1[\text{OH}^-]} + \frac{1}{k_1 K_1}\right)\frac{1}{[\text{Trp}]} + \frac{1}{k_1} \tag{11}$$

$$\frac{1}{k_U} = \left(\frac{1}{k_1 K K_1[\text{Trp}]}\right)\frac{1}{[\text{OH}^-]} + \frac{1}{k_1 K_1[\text{Trp}]} + \frac{1}{k_1} \tag{12}$$

According to Eq. (11), a plot of $1/k_U$ versus 1/[Trp] at constant [$OH^-$] should be linear with a positive intercept. This is verified in Fig. 7. The intercept corresponds to $1/k_1$, from which the value of $k_1$ of $15.1 \times 10^{-4}\ s^{-1}$ at 25 °C is determined. Similarly, on the basis of Eq. (12), the plot of $1/k_U$ versus 1/[$OH^-$] at a constant substrate concentration yields a straight line with slope and intercept equal to $1/k_1 K K_1[\text{Trp}]$ and $1/k_1 K_1[\text{Trp}]$, respectively. Now, with the help of the slope and intercept of such plot, the calculated values of $K$ and $K_1$ at 25 °C were 2.46 and 112.99 $dm^3\ mol^{-1}$, respectively.

## 5. Mechanism of the copper(II)-catalyzed oxidation reaction

In aqueous alkaline media [33], copper(II) forms a tetrahydroxycuprate(II) complex, $[Cu(OH)_4]^{2-}$. Also, it is reported [34] that copper(II) acts as an efficient catalyst in some redox reactions involving amino acids, particularly in alkaline media. This behavior is due to the presence of both carboxylate and amine groups in the amino acids, which may act as nucleophiles, depending on the pH of the medium.

The reaction between HCF and L-tryptophan in alkaline medium in the presence of small amounts of copper(II) catalyst is similar to the uncatalyzed reaction with respect to the stoichiometry, reaction orders as well as the influence of both ionic strength and dielectric constant of the medium. The reaction was fractional-first order with respect to the copper(II) catalyst. The less than unit orders observed with respect to both [Trp] and [$Cu^{II}$] presumably result from a complex formation between the L-tryptophan substrate and copper(II) catalyst in a pre-equilibrium step before the reaction with the oxidant. Spectroscopic evidence for complex formation between the oxidant and substrate was also obtained from UV–Vis spectra shown in Fig. 1(b). Furthermore, the formation of the complex was proved kinetically by the non-zero intercept of the [$Cu^{II}$]/$k_C$ versus 1/[Trp] plot (Fig. 8). Such complexes between L-tryptophan and copper(II) catalyst have been reported in earlier studies [35–38].

In view of the abovementioned aspects, deprotonated L-tryptophan is suggested to combine with a $Cu^{II}$ species, $[Cu(OH)_4]^{2-}$, to form a complex ($C_2$) prior to the rate-determining step. The oxidant HCF then attacks this complex in the rate-determining step to form L-tryptophan free radical and HCF(II), with regeneration of the catalyst $Cu^{II}$. This is subsequently followed by fast steps that give rise to the final oxidation products, as shown in Scheme 2.

An alternative reaction mechanism [39–41] for metal ion-catalyzed oxidation may be proposed. It involves the formation of an intermediate complex ($C_2$) between the metal ion catalyst and the amino acid that has on further interaction with the oxidant in the rate-determining step yields another complex ($C_3$) of a higher valence metal ion and the reduced

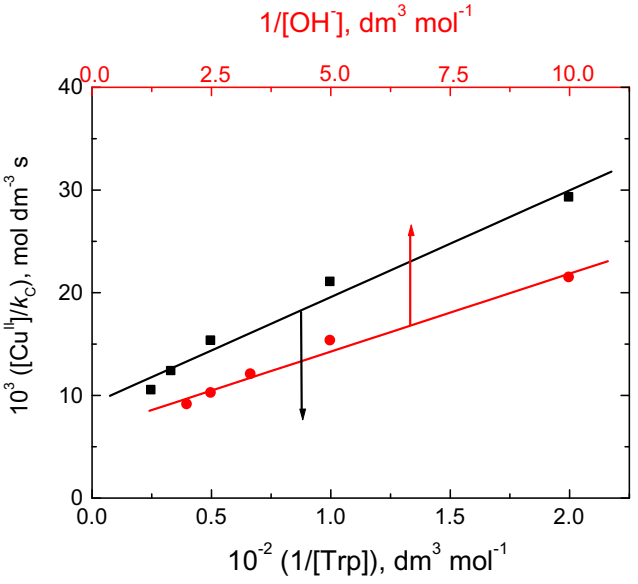
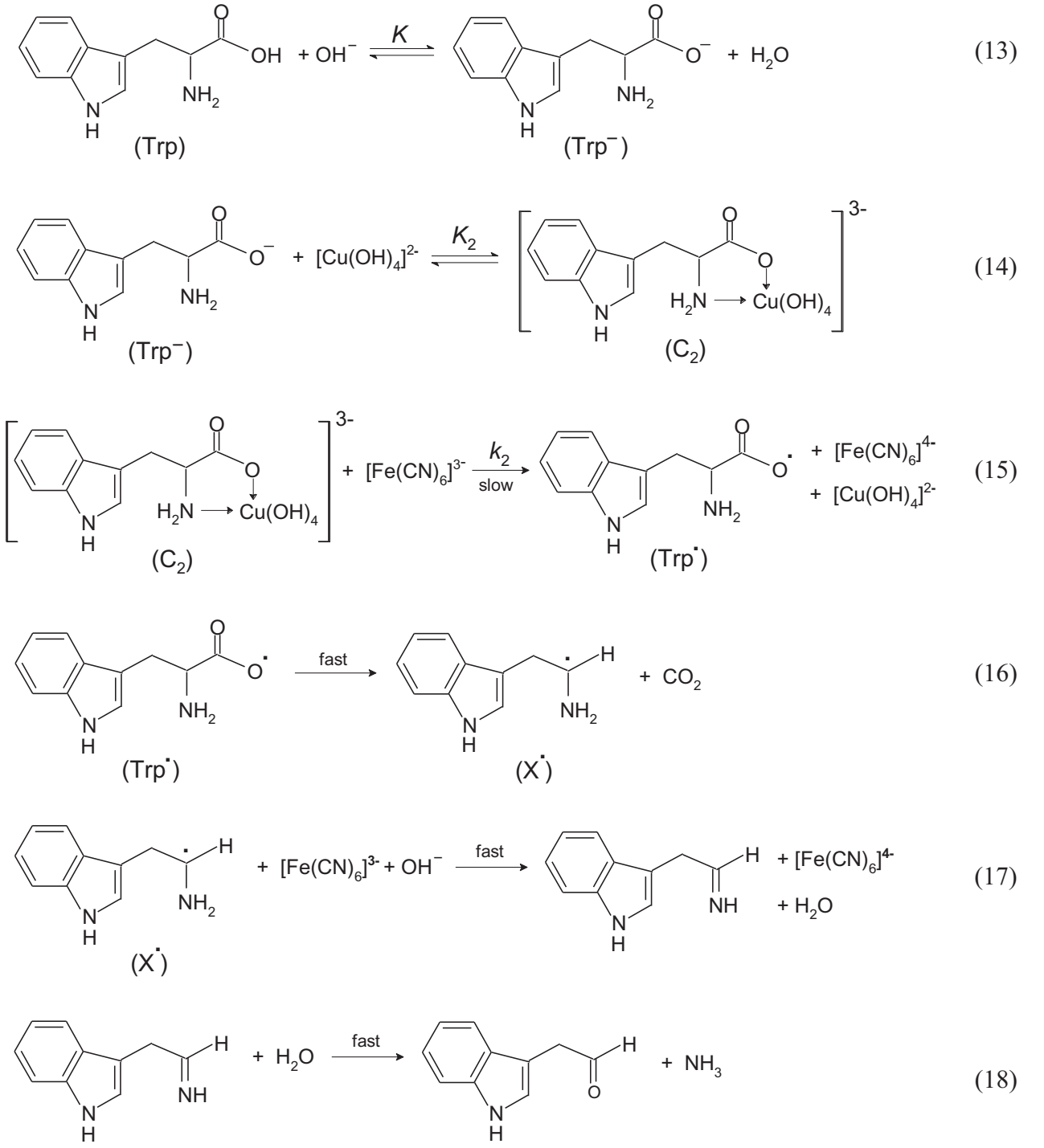



**Figure 8** Verification of Eqs. (29) and (30) in the copper(II)-catalyzed oxidation of L-tryptophan by hexacyanoferrate(III) in alkaline medium. [HCF] = $7.0 \times 10^{-4}$ and $I$ = 0.5 mol $dm^{-3}$ at 25 °C.




**Scheme 2** Mechanism of copper(II)-catalyzed oxidation of L-tryptophan by hexacyanoferrate(III) in alkaline medium.

form of the oxidant. Such a complex is rapidly decomposed to give rise to the intermediate radical with regeneration of the catalyst, subsequently followed by fast steps to yield the final oxidation products, as illustrated in Scheme 3.

The suggested mechanism leads to the following rate law,

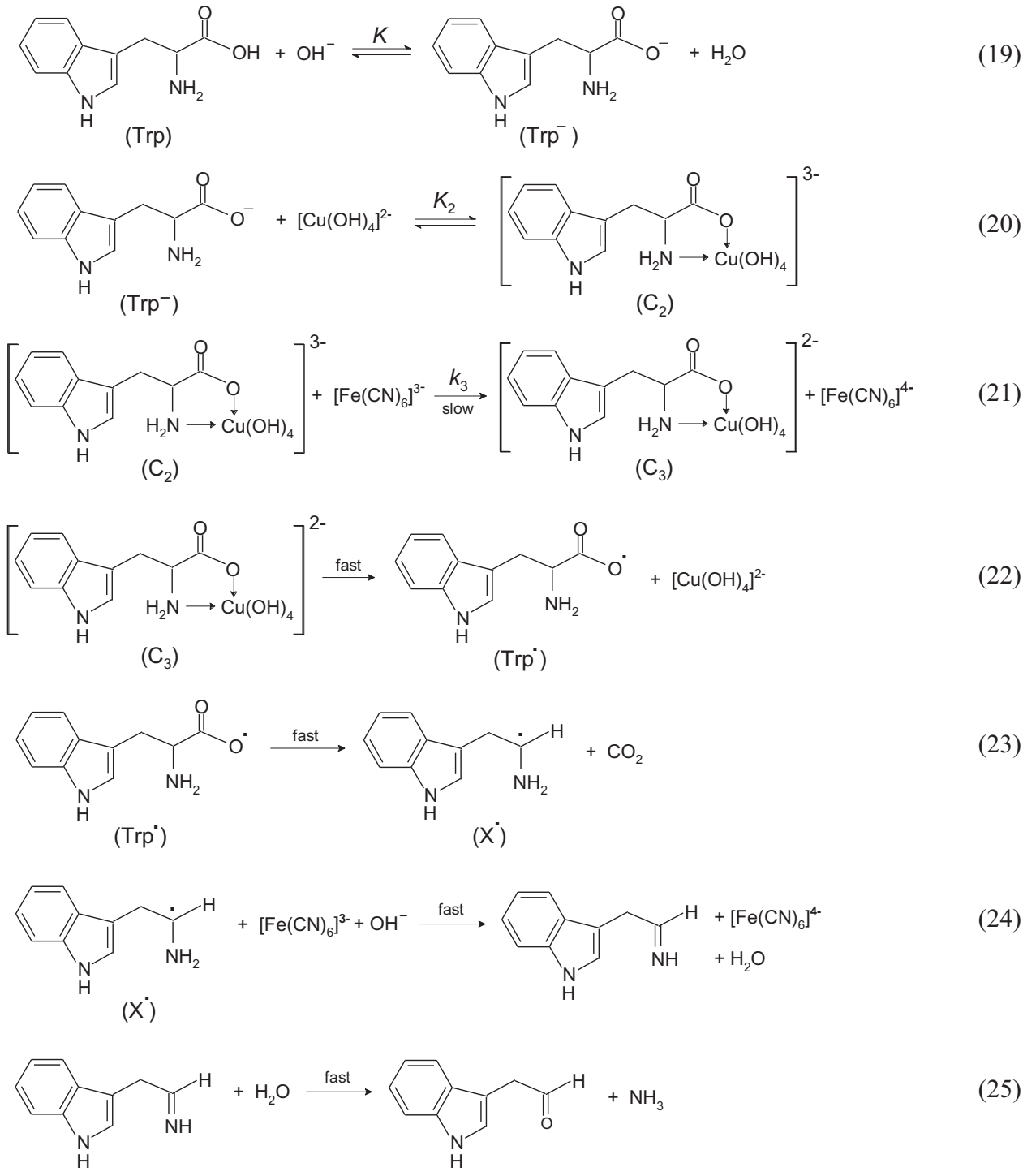
$$\text{Rate} = \frac{k_2 K K_2 [\text{Trp}][\text{HCF}][\text{Cu}^{\text{II}}][\text{OH}^-]}{1 + K[\text{OH}^-] + K K_2 [\text{Trp}][\text{OH}^-]} \tag{26}$$

Also, the above rate law is consistent with all observed orders with respect to different species.

Under a pseudo-first order condition, the rate-law can be expressed by Eq. (27),

$$\text{Rate} = \frac{-d[\text{HCF}]}{dt} = k_C[\text{HCF}] \tag{27}$$

Comparing Eqs. (26) and (27), the following relationship is obtained,




**Scheme 3** An alternative mechanism of copper(II)-catalyzed oxidation of L-tryptophan by hexacyanoferrate(III) in alkaline medium.

$$k_C = \frac{\text{Rate}}{[\text{HCF}]} = \frac{k_2 K K_2 [\text{Trp}][\text{Cu}^{\text{II}}][\text{OH}^-]}{1 + K[\text{OH}^-] + KK_2[\text{Trp}][\text{OH}^-]} \quad (28)$$

Eq. (28) can be rearranged to the following forms, which are suitable for verification,

$$\frac{[\text{Cu}^{\text{II}}]}{k_C} = \left(\frac{1}{k_2 K K_2 [\text{OH}^-]} + \frac{1}{k_2 K_2}\right)\frac{1}{[\text{Trp}]} + \frac{1}{k_2} \quad (29)$$

$$\frac{[\text{Cu}^{\text{II}}]}{k_C} = \left(\frac{1}{k_2 K K_2 [\text{Trp}]}\right)\frac{1}{[\text{OH}^-]} + \frac{1}{k_2 K_2 [\text{Trp}]} + \frac{1}{k_2} \quad (30)$$

Eqs. (29) and (30) require that plots of $[\text{Cu}^{\text{II}}]/k_C$ versus $1/[\text{Trp}]$ at constant $[\text{OH}^-]$ and $[\text{Cu}^{\text{II}}]/k_C$ versus $1/[\text{OH}^-]$ at constant [Trp], respectively, are linear with positive intercepts on the $[\text{Cu}^{\text{II}}]/k_C$ axes. These requirements are verified in Fig. 8. Similarly to the uncatalyzed reaction, values of $k_2$, $K$ and

$K_2$ at 25 °C are calculated from the slopes and intercepts of the aforementioned plots to be 111.07 mol $dm^{-3}$ $s^{-1}$, 5.48 $dm^3$ $mol^{-1}$ and 136.51 $dm^3$ $mol^{-1}$, respectively.

## 6. Conclusions

A comparative study of uncatalyzed and copper(II)-catalyzed oxidation of L-tryptophan by hexacyanoferrate(III) in alkaline medium was performed. The rate of copper(II)-catalyzed reaction are about fourfold faster than that of the uncatalyzed reaction. Activation parameters are evaluated for both reactions. The overall sequences described here are consistent with all experimental findings.

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## Appendix A. Derivation of rate law for uncatalyzed reaction

According to the proposed mechanistic Scheme 1,

$$\text{Rate} = \frac{-d[\text{HCF}]}{dt} = k_1[\text{C}_1] \tag{A1}$$

$$K = \frac{[\text{Trp}^-]}{[\text{Trp}][\text{OH}^-]}, \quad [\text{Trp}^-] = K[\text{Trp}][\text{OH}^-] \tag{A2}$$

and

$$K_1 = \frac{[\text{C}_1]}{[\text{Trp}^-][\text{HCF}]}, \quad [\text{C}_1] = K_1[\text{Trp}^-][\text{HCF}] = KK_1[\text{Trp}][\text{HCF}][\text{OH}^-] \tag{A3}$$

Substituting Eq. (A3) into Eq. (A1) leads to,

$$\text{Rate} = k_1KK_1[\text{Trp}][\text{HCF}][\text{OH}^-] \tag{A4}$$

The total concentration of Trp is given by,

$$[\text{Trp}]_T = [\text{Trp}] + [\text{Trp}^-] + [\text{C}_1] \tag{A5}$$

where '*T*' stands for total concentration.

Substituting Eqs. (A2) and (A3) into Eq. (A5), and rearrangement gives,

$$[\text{Trp}]_T = [\text{Trp}] + K[\text{Trp}][\text{OH}^-] + KK_1[\text{Trp}][\text{HCF}][\text{OH}^-] \tag{A6}$$

$$[\text{Trp}]_T = [\text{Trp}](1 + K[\text{OH}^-] + KK_1[\text{HCF}][\text{OH}^-]) \tag{A7}$$

Therefore,

$$[\text{Trp}] = \frac{[\text{Trp}]_\text{T}}{1 + K[\text{OH}^-] + KK_1[\text{HCF}][\text{OH}^-]} \tag{A8}$$

In view of the low [HCF], the third denominator term, $KK_1$[HCF][OH], in the above equation can be neglected. Therefore,

$$[\text{Trp}]F = \frac{[\text{Trp}]_\text{T}}{1 + K[\text{OH}^-]} \tag{A9}$$

Also,

$$[\text{HCF}]_T = [\text{HCF}] + [\text{C}_1] \tag{A10}$$

Substituting Eq. (A3) into Eq. (A10) gives,

$$[\text{HCF}]_T = [\text{HCF}](1 + KK_1[\text{Trp}][\text{OH}^-]) \tag{A11}$$

$$[\text{HCF}] = \frac{[\text{HCF}]_\text{T}}{1 + KK_1[\text{Trp}][\text{OH}^-]} \tag{A12}$$

In view of the concentration of [$OH^-$],

$$[\text{OH}^-] = [\text{OH}^-]_T \tag{A13}$$

Substituting Eqs. (A9), (A12) and (A13) into Eq. (A4) (and omitting 'T' subscript) gives,

$$\text{Rate} = \frac{k_1KK_1[\text{Trp}][\text{HCF}][\text{OH}^-]}{(1 + K[\text{OH}^-])(1 + KK_1[\text{Trp}][\text{OH}^-])} \tag{A14}$$

Under pseudo-first order condition, the rate-law can be expressed by Eq. (A15),

$$\text{Rate} = \frac{-d[\text{HCF}]}{dt} = k_\text{U}[\text{HCF}] \tag{A15}$$

Comparing Eqs. (A14) and (A15), the following relationship is obtained,

$$K_\text{U} = \frac{k_1KK_1[\text{Trp}][\text{OH}^-]}{(1 + K[\text{OH}^-])(1 + KK_1[\text{Trp}][\text{OH}^-])} \tag{A16}$$

$$k_\text{U} = \frac{k_1KK_1[\text{Trp}][\text{OH}^-]}{1 + K[\text{OH}^-] + KK_1[\text{Trp}][\text{OH}^-] + K^2K_1[\text{Trp}][\text{OH}^-]^2} \tag{A17}$$

In view of the low concentration of Trp used, the term $K^2K_1$[Trp][$OH^-$] in the fourth denominator of Eq. (A17) is negligibly small compared to unity. Therefore, Eq. (A17) can be written as,

$$k_\text{U} = \frac{k_1KK_1[\text{Trp}][\text{OH}^-]}{1 + K[\text{OH}^-] + KK_1[\text{Trp}][\text{OH}^-]} \tag{A18}$$

The rate law for the copper(II)-catalyzed reaction was also derived similarly.

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